for color development before reading in a Klett-Summerson colorimeter, using a 540 filter. The instrument was set at 0 with distilled water. A phosphatase unit is the mg of tyrosine equivalent to the phenol liberated from the substrate per g dry weight of tissue per hr. A standard curve was prepared with L-tyrosine according to Greenberg, Lucia, and Weitzman (*5*).

The activities of AcP-ase and AlP-ase of seminal vesicle tissue at various pH values is shown in Fig. 1.

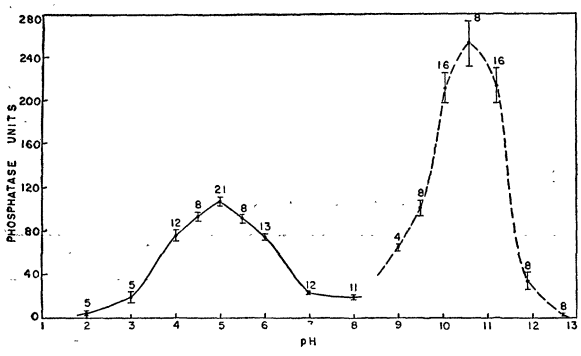

FIG. 1. Phosphatase activity of rat seminal vesicles as influenced by pH. Phosphatase units expressed as means and SE. Number of animals used for each pH value is indicated above mean.

Maximum AcP-ase activity was obtained at pH 5.0, whereas maximum for AlP-ase was at 10.6. It is significant that within the pH range usually associated with intracellular functions the activities of these enzymes are considerably less than their maxima.

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Differentiation of Nucleic Acids and Acid Mucopolysaccharides in Histologic Sections by Selective Extraction with Acids[1]

William B. Atkinson[2]

Department of Anatomy, College of Medicine, University of Cincinnati, Cincinnati, Ohio

The markedly basophilic substances in sections of fixed tissues prepared by the paraffin technique fall into two principal groups, nucleic acids and acid mucopolysaccharides. The affinity of the former for basic dyes is attributable to their phosphoric acid moiety, whereas the staining of the latter is due to their component sulfuric or glucuronic acid (*1*). The histochemical methods devised for the microscopic demonstration of these substances have been based on one of two principles: identification by properties other than basophilia—e.g., the Feulgen nucleal reaction for desoxyribonucleic acid; or the use of basic dyes in conjunction with the differential extraction of various of the stainable substances. The present investigation is concerned only with the latter method.

The first tinctorial method developed for the histochemical demonstration of RNA was based on its removal from tissue sections by the enzymatic action of relatively crude preparations of ribonuclease (*2*). Sites of RNA were determined by comparing the distribution of stainable material in parallel digested and undigested sections. Although the technique was refined by subsequent investigators (*3*), it has remained of limited usefulness because of the difficulties involved in the preparation of ribonuclease and because of the high cost of the commercial enzyme. No satisfactory method has been devised for the removal of acid mucopolysaccharides as a group, although the preliminary results obtained with pectinases (*4*) warrant further investigation. The distribution of hyaluronic acid has been demonstrated successfully in a number of mammalian tissues by the use of hyaluronidase (*5*).

The disadvantages of the enzymatic method for the extraction of RNA from tissue sections appeared to have been eliminated by the recent introduction of perchloric acid ($HClO_4$) (*6–8*), based on the procedure developed by Ogur and Rosen (*9, 10*) for the separation of RNA from DNA components in the residue of plant and animal tissue homogenates after the removal of alcohol- and acid-soluble compounds and phospholipids. The substitution of $HClO_4$ for ribonuclease in the differentiation of RNA from DNA in paraffin sections seems to be justified. However, the effect of $HClO_4$ on the subsequent stainability of acid mucopolysaccharides and, therefore, the value of the reagent in distinguishing cytoplasmic RNA from these substances have not been considered. Neither has the efficiency of $HClO_4$ been compared with that of hydrochloric acid and trichloracetic acid

TABLE 1

EFFECT OF FIXATION ON EXTRACTION OF RNA FROM LIVER

(*N* $HClO_4$ at 5° C for 18 hr)

Fixative	Untreated	H_2O	Cytoplasmic RNA	Chromatin
Zenker's	+++	+++	+++	+++
Carnoy's	+++	+++	+	+++
Ca-formal	+++	+++	+	+++
80% Ethanol	+++	+++	±	+++
PAF	+++	+++	±	+++

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(CCl_3COOH), which have also been used to extract nucleic acids from fixed tissues (*11, 12*). The present experiments were designed, therefore, to determine the relative extractability of RNA, DNA, and several representative acid mucopolysaccharides from paraffin sections of fixed tissues by treatment with normal solutions of HCl, $HClO_4$, and CCl_3COOH under various conditions of time and temperature.

The tissues selected as test material were chosen because the morphologic distribution of their component nucleic acids and acid mucopolysaccharides has been well established by previous histochemical studies. The parenchymal cells of rat and mouse liver and rat pancreas were used for cytoplasmic RNA, the chromatin of rat lymphocytes for DNA. Representative acid mucopolysaccharides were provided by the mast cell granules of the rat (heparin), the matrix of the tracheal cartilages of the rat (chondroitin sulfate), and the mucus of the human uterine endocervix (mucoitin sulfate). The mouse liver was fixed in 80% ethyl alcohol, calcium chloride-formalin, alcoholic picro-formalin (PAF), Carnoy's fluid, and Zenker's fluid; the remaining tissues were fixed in PAF. The specimens were embedded in paraffin. Sections were cut at 6 μ and were mounted on glass slides by means of a dilute solution of Mayer's albumen in distilled water. Treated and untreated sections were stained for 1 hr at room temperature in 0.01 *M* toluidine blue in

TABLE 2

EXTRACTION OF PAF-FIXED BASOPHILIC SUBSTANCES BY NORMAL ACIDS

Agent	Temp (°C)	Time	Cytoplasmic RNA		Lymphocyte chromatin	Acid mucopolysaccharide		
			Liver	Pancreas		Mucus	Mast cells	Cartilage
None	—	—	+++	+++	+++	+++	+++	+++
H_2O	5	30 hr	+++	+++	+++	+++	+++	+++
HCl	5	18 "	++	+++	+++	+++	+++	+++
	5	24 "	+	++	+++	++	+++	+++
	5	30 "	±	++	+++	+	++	+++
$HClO_4$	5	18 "	+	+	+++	+	++	+++
	5	24 "	±	+	+++	±	++	+++
	5	30 "	0	±	+++	±	++	++
CCl_3COOH	5	18 "	+++	+++	+++	+++	+++	+++
	5	24 "	+++	+++	+++	+++	+++	+++
	5	30 "	+++	+++	+++	+++	+++	+++
H_2O	37	2 "	+++	+++	+++	+++	+++	+++
HCl	37	½ "	+	++	+++	+	+++	+++
	37	1 "	0	+	+++	0	+++	+++
	37	2 "	0	0	++	0	+++	+++
$HClO_4$	37	½ "	0	+	+++	+	+	+++
	37	1 "	0	+	++	±	+	++
	37	2 "	0	±	++	0	+	++
CCl_3COOH	37	½ "	++	++	+++	+	++	+++
	37	1 "	±	+	+++	±	++	+++
	37	2 "	0	0	++	0	+	+++
H_2O	60	15 min	±	++	+++	+	+++	+++
	60	30 "	±	+	+++	±	+++	+++
	60	45 "	0	+	++	±	+++	+++
HCl	60	15 "	0	0	+++	0	+++	+++
	60	30 "	0	0	Irregular	0	++	++
	60	45 "	0	0	0	0	++	++
$HClO_4$	60	15 "	0	0	Irregular	0	+	+++
	60	30 "	0	0	"	0	±	++
	60	45 "	0	0	"	0	±	++
CCl_3COOH	60	15 "	0	0	+++	0	+	+++
	60	30 "	0	0	++	0	±	++
	60	45 "	0	0	Irregular	0	±	++
H_2O	90	5 "	+++	+++	+++	++	+++	+++
	90	10 "	+++	+++	+++	+	+++	+++
	90	15 "	+++	+++	+++	+	+++	+++
HCl	90	5 "	0	0	0	0	++	++
	90	10 "	0	0	0	0	+	+
	90	15 "	0	0	0	0	0	0
$HClO_4$	90	5 "	0	0	Irregular	0	0	+
	90	10 "	0	0	±	0	0	0
	90	15 "	0	0	0	0	0	0
CCl_3COOH	90	5 "	0	0	Irregular	0	0	++
	90	10 "	0	0	±	0	0	+
	90	15 "	0	0	0	0	0	+

0.1 *M* citrate buffer at pH 4.5. After being rinsed in distilled water, the stained sections were dehydrated in ethyl alcohol and mounted in xylene-damar .

Normal solutions of HCl, $HClO_4$, and CCl_3COOH were prepared from C.P. reagents by electrometric titration with *N* NaOH. The pH of the normal acids at room temperature was 0.05, 0.00, and 0.29, respectively. Mounted tissue sections were incubated in each of the acids and in distilled water under various conditions of time and temperature prior to staining. The basophilia of each of the tissue components under study was then compared with that exhibited in stained sections that had not been incubated in either acid or distilled water. It should be noted that the depth of staining of each component in untreated sections was recorded as "three plus" (+++), although in reality the basophilia of the different substances varied in intensity. The experimental conditions and observations are summarized in Tables 1 and 2.

It can be readily seen from Table 1 that the method of fixation considerably alters the extractability of RNA by cold $HClO_4$. This observation explains the failure of the method when applied to paraffin sections of Zenker-fixed tissue (*8*) after its initial successful use with alcohol-fixed material (*6*). The increased acid-resistance of RNA in paraffinized fixed tissues, however, is due in large part to the embedding process, since RNA is readily removed from frozen sections of Zenker-fixed material (*8*). The present data concerning the effects of time and temperature on the action of $HClO_4$ on the stainability of nucleic acids (Table 2) are in substantial agreement with the observations of previous investigators in that (1) the extractability of RNA varies in different tissues, (2) the rate of extraction increases with temperature, and (3) at elevated temperatures both RNA and DNA may be completely removed from the sections. In addition, the present studies demonstrate that the stainability of acid mucopolysaccharides is similarly affected. The ready extractability of epithelial mucus, in particular, precludes the use of this method on tissues which may contain both cytoplasmic RNA and mucin; e.g., vaginal epithelium, anterior lobe of the pituitary, salivary glands, etc. At present, then, it would be advisable to use the ribonuclease technique under such circumstances.

The results obtained with HCl and CCl_3COOH are, in general, qualitatively similar to those obtained with $HClO_4$, although under the same conditions of time and temperature the amounts of basophilic material extracted by the three acids differed. It is of interest to note that DNA, heparin, and chondroitin sulfate apparently are hydrolyzed at a slower rate in HCl than in either $HClO_4$ or CCl_3COOH (most evident at 37° C), whereas HCl is almost equally as effective as $HClO_4$ in removing RNA. This property of HCl should be advantageous in the differentiation of RNA from DNA, but the ready solubility of epithelial mucus may limit the wider application of the method as noted above.

The present experiments have provided specific data on the acid-extractability of but a few representative acid mucopolysaccharides and nucleic acids in PAF-fixed mammalian tissues. The results, however, indicate that HCl, $HClO_4$, and CCl_3COOH are nonspecific in action and may remove members of both these groups of basophilic compounds, depending on the circumstances. In the histochemical differentiation of RNA from DNA, acid extraction may be substituted for ribonuclease digestion only in the absence of extractable acid mucopolysaccharides and only when the conditions of time and temperature are empirically determined for the particular organ to be studied and fixative used.

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The Accuracy and Convenience of Silicone-treated Microliter Pipettes

Edward L. Duggan and Kendric C. Smith

Department of Biochemistry, University of California School of Medicine, Berkeley

The present deserved popularity of paper chromatographic techniques has resulted in the widespread use of microliter pipettes, with occasional misunderstanding of their proper usage or inherent accuracy. Existing methods of calibration (*1*) of pipettes of less than 100 µl capacity are based upon the weight of mercury contained in the dry pipette. Thus, such pipettes are capable of accurate and reproducible content but not of delivery.

The advent of silicone mixtures (*2, 3*) that may be applied to general glassware to produce water-repellent surfaces promises great improvement in quantitative techniques, along the lines indicated by Gilbert (*3*). This paper enumerates several advantages which accrue to microliter chemistry by the use of water-repellent coatings.

To test the efficacy of such coatings in permitting the use of 10-µl pipettes for delivery, two such pipettes (Microchemical Specialties No. 280B) were treated with Desicote according to the procedure outlined in the manufacturer's bulletin (*2*).